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Formulation and evaluation of nanobiotherapeutics of *Terminalia arjuna* through plant tissue culture for atherosclerosis

Pradnya Pradeep Wadekar^{1,2*}  and Vijay Rajaram Salunkhe³

Abstract

Background The study seeks to investigate the therapeutic potential of *Terminalia arjuna* callus in addressing atherosclerosis. In order to get maximum beneficial phytoconstituents from *Terminalia arjuna*, it is recommended to harvest the bark from Arjuna trees that are at least 15 years old and a gap of minimum 2 years should be kept before harvesting bark from the same plant. The callus culture technique was employed to expedite the process. The callus culture extract was subsequently converted into a nanosuspension with the aim of improving the efficacy of its phytoconstituents. It was then subjected to a comprehensive series of in vitro and in vivo evaluations to ascertain its potential for treatment of atherosclerosis.

Results Liquid chromatography–mass spectrometry analysis of the callus extract confirmed the presence of flavonoids and terpenoids, known for their antioxidant and anti-inflammatory activities. Some terpenoids were even absent in Arjuna tree naturally. TEM images validated successful entrapment of the extract within the nanoparticles. In vitro analysis for antilipase and antioxidant assay confirmed the antiatherosclerotic potential of the extract. In vivo tests on rat blood serum demonstrated a significant reduction in total cholesterol, low-density lipoprotein, triglycerides, high-density lipoprotein, and very low-density lipoprotein. Histopathological analysis of rat aortas showed additional confirmation of antiatherosclerotic action.

Conclusion In conclusion, the study highlights the potential of nanosuspension derived from *Terminalia arjuna* callus extract as a comprehensive therapeutic strategy for atherosclerosis treatment. The research highlights antioxidant, anti-inflammatory, and antiatherosclerotic properties of the callus, hinting at its viability as a potential treatment for atherosclerosis. This interdisciplinary investigation emphasizes the promising role of traditional medicinal plants within modern medical paradigms.

Keywords Arjuna bark, Atherosclerosis, Nanosuspension, Callus culture

Background

Atherosclerosis is a chronic inflammatory condition resulting in cardiovascular disease with life-threatening implications. Atherosclerosis is a complex and multifaceted condition marked by the accumulation of plaque within arteries. This can lead to reduced blood flow, contributing to a range of cardiovascular diseases (CVDs). CVDs are one of the leading causes of death worldwide, and their prevalence is rapidly increasing, owing in large part to changes in lifestyle and an increase in the elderly population [1]. Since atherosclerosis is a

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complex condition affected by numerous risk factors, there are various well-established and innovative strategies to diagnose and manage this disease. During the early phases of atherosclerosis, the primary objective of treatment is on managing significant risk factors such as elevated cholesterol levels, hypertension, and elevated blood sugar. Making alterations to one's diet, engaging in regular physical activity, and quitting smoking are all assumed to play pivotal roles in both the prevention and management of atherosclerosis. The current treatment for management of atherosclerosis includes drugs that have antihypercholesterolemic, antihypertensive, hypoglycemic, and antiplatelet activity. Anti-inflammatory drugs like canakinumab, methotrexate, and colchicine have passed clinical trials but need to be further assessed for their therapeutic efficacy. The typical medications utilized to address hypercholesterolemia are statins solely or in combination with drugs like ezetimibe. These drugs function by inhibiting the hepatic enzyme HMG CoA reductase, a crucial enzyme in the synthesis of cholesterol. The primary advantage of statins lies in their ability to lower low-density lipoproteins (LDL). However, they suffer from adverse effects mainly statin-associated muscle symptoms, myalgias, and glucose intolerance. Additionally, the utilization of statins has been associated with the development of type 2 diabetes mellitus [2–4].

Considering the negative effects of synthetic medications, emphasis should be given to embrace natural drugs, which have negligible adverse reactions. Natural origin drugs still continue to be the focus of pharmaceutical research because substitute drug discovery methods have failed to provide lead compounds for treatment of various diseases like metabolic diseases. *Terminalia arjuna*, also known as Arjuna in Hindi, belongs to the family Combretaceae and is found in India, Mauritius, and Sri Lanka. Arjuna has been documented in numerous ancient Indian medical texts, including Charaka Samhita, Astang Hridayam, and Sushruta Samhita, dating back to the Vedic period. Notably, it was Vagabhatta who first recommended the utilization of stem bark powder for heart-related conditions. Ayurvedic practitioners have employed this plant in the management of cardiovascular ailments [5]. The stem bark, fruits, and even leaves of Arjuna are commonly used in different traditional systems across India for various medicinal properties such as astringent, demulcent, expectorant, styptic, antidiysenteric, cardiotonic, and urinary astringent. It is also used in the treatment of ulcers, earaches, and even bark ashes are used for snakebites and scorpion stings [6]. Numerous experimental and clinical studies have effectively showcased Arjuna's potential as an anti-ischemic agent, antiatherogenic agent, and a strong antioxidant. As per data shared for Scientific Harvesting—Dos and

Don'ts by NTFP Centre of Excellence (NCE) established by Government of Tripura, India; the bark of Arjuna tree should not be harvested before 15 years of growth to get quality product with a minimum gap of 2 years for successive harvesting [6–11].

The present study includes the callus culture to reduce the harvesting time period. Nanoparticle delivery systems offer the enhanced stability and solubility of encapsulated drug molecules, facilitating their movement across membranes, and extending circulation time to enhance both safety and effectiveness of the medicament [12, 13]. In this study, a multidisciplinary approach was employed to create a nanosuspension from Arjuna callus.

Methods

Preparation of callus

The branch of Arjuna was collected from Dr. Babasaheb Ambedkar Central Nursery Kagal, India, in the month of June, and plant was authenticated form Botanical Survey of India, Pune. The leaves were surface sterilized. Murashige and Skoog agar media with different concentrations of 2, 4-dichlorophenoxy acetic and kinetin were used for callus induction. The explants were incubated at 25 ± 2 °C under 16-h photo-period of 2000 lx with white light. The relative humidity was maintained at 60–70% during incubation at Seem Biotech Pvt Ltd, Warananagar, India. Sub-culturing was carried out every six weeks. After 6 months, the callus cultures were weighed and callus index was calculated.

Extraction of callus

The callus culture obtained was dried at 50 ± 5 °C in hot air oven and was subjected to ethanolic extraction using Soxhlet apparatus and subsequently dried in vacuum dryer.

Preparation of arjuna callus extract nanosuspension (ACEN)

One gram of extract was mixed with 20 ml ethanol. The solution was injected drop wise in 100 ml aqueous solution of polyvinyl pyrrolidone K30 (PVP K30) and was subjected to mechanical stirring for 6 h. A factorial design was employed, taking into account two independent factors: the concentration of PVP K30 and mixing speed, each varying at three different levels.

Evaluation of nanosuspension

Bruker, ALPHA Infrared Spectrophotometer was used for checking the compatibility of callus extract with the PVP K30. The Horiba Zetasizer was used for determination of particle size and zeta potential [14]. Absorption maxima were determined using a Jasco V-750 UV Spectrophotometer from Japan to calculate % drug content,

% drug encapsulation, and % drug release. For the % drug release study, a dialysis bag (dimensions: flat width 28.46 mm, inflated diameter 18 mm; provided by HiMedia Laboratories Pvt, Ltd) was utilized. One milliliter of 10 µg/ml of each ACEN batch was placed in a dialysis bag immersed in 300 mL of diffusion media (phosphate buffer saline—PBS, pH 7.4) in USP Type I dissolution test apparatus. Samples were withdrawn at 5-min intervals over 40 min from the receptor compartment and

solution with 100 µL of a 0.1% methanolic DPPH solution. Similarly, test samples were prepared by mixing different concentrations of 100 µL of an aqueous callus extract solution with 100 µL of the 0.1% methanolic DPPH solution. After mixing, all samples were incubated for 30 min in a dark environment at room temperature. The absorbance was recorded three times at 490 nm [18, 19].

The radical scavenging activity was determined using the following formula:

$$\% \text{ radical scavenging activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test sample}}{\text{Absorbance of Control}} \times 100$$

analyzed at 282 nm. The % drug content, % drug encapsulation efficiency, and % drug release studies were conducted in triplicate [15].

Acceleration stability studies for the optimized batch were carried out according to ICH guidelines for 90 days. It involved a modification in the intermediate storage condition, transitioning from 30 °C ± 2 °C and 60% ± 5% relative humidity (RH). The adequate amount of freeze-dried optimized ACEN batch was subjected to acceleration stability study. The particle size, % drug content, and % drug release study were used as parameters to check the stability of ACEN [16].

Antilipase assay

The enzymatic activity of porcine pancreatic lipase (PPL, type II) was assessed using p-nitrophenyl butyrate (p-NPB) as a substrate. PPL stock solution (1 mg/mL) was prepared in 0.1 mM potassium phosphate buffer (pH 6.0) and stored at -20 °C. Different concentrations 20, 40, 60, 80, and 100 µg/mL of extract and standard drug Orlistat were pre-incubated with PPL for 1 h in a 0.1 mM potassium phosphate buffer (pH 7.2), supplemented with 0.1% Tween 80, at 30 °C. After addition of 0.1 µL of p-NPB, all samples were incubated at 30 °C for 5 min. The absorption for all samples was recorded at 405 nm. The analysis was performed three times for each concentration of sample and standard. The inhibitory activity (I) was calculated using the following formula [17]:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Experimental animals

The protocol was approved by the Institutional Animal Ethics Committee of Appasaheb Birnale College of Pharmacy, Sangli (Approval number-IAEC/ABCP/13/2021–22). Healthy young male Wistar rats aged 6–8 weeks weighing approximately 200–250 g were divided into four groups, with six rats in each group. Prior to the initiation of the experimental procedure, one-week acclimatization period maintaining a temperature of 24 ± 3 °C, a humidity level of 50–60% RH, and a 12-h light/dark cycle, was provided for the rats.

Acute toxicity study

An acute toxicity assessment was conducted on rats in accordance with the OECD guideline 423 using the up-and-down procedure. Three nulliparous and non-pregnant female rats aged 8 to 12 weeks were employed for the study. The rats were fasted overnight before dosing, which extended for an additional 3-h post-dosing. Close monitoring of the animals was carried out for the initial 24 h to detect any signs of toxicity and continued for 72 h to identify potential mortalities. The determined LD50 value was 3000 mg/kg of rat body weight. As a result, a dose of 300 mg/kg body weight was selected for ACEN administration [4, 7, 11].

Antioxidant activity assay

The antioxidant potential of the nanosuspension was assessed through its ability to scavenge free radicals using DPPH (1,1-diphenyl-2, picryl-hydrazyl) radicals. The standard solutions were prepared by mixing different concentrations of 100 µL of aqueous ascorbic acid

Preparation of high-fat diet (HFD)

The fine powder of the conventional standard animal pellets was combined with 2% cholesterol, 1% cholic acid, 40% sucrose, and 10% coconut oil. The resulting powdered mixture was blended with an appropriate amount

of water to form compact feed spheres. These spheres were then stored in a refrigerator.

Animal test groups

The animals were randomly distributed into four distinct groups, each group comprising of six rats. Group I, designated as normal group, was provided with the standard rat animal pellets. Group II, known as the disease control group, was fed a HFD. Throughout the study, both Group I and Group II received normal saline per day orally. Group III, referred to as the Standard group, received HFD and atorvastatin orally at a dosage of 10 mg/kg body weight of rat per day, while Group IV received HFD and ACEN at a dose of 300 mg/kg body weight per day orally. Upon the conclusion of the 30-day study period, the rats were fasted overnight. Subsequently, the rats were anesthetized using diethyl ether, and blood samples were collected from the retro orbital venous plexus. These blood samples were promptly transferred to sterile plain tubes to conduct biochemical tests [20–22].

Atherogenic index

The atherogenic index of serum (AIS) is the measure of the extent of atherosclerotic lesions based on serum lipids. The atherogenic index was calculated using following formula [23]:

$$\text{AIS} = \frac{\text{TC} - \text{HDL}}{\text{HDL}}$$

where TC=concentration of total cholesterol in mg/ml.
HDL=concentration of HDL in mg/ml.

Histology of the aorta

The rats were killed by cervical decapitation. Their aortas were dissected out carefully. The aortas were stored in 10% formaline solution and sent to a local pathological laboratory for hematoxylin and eosin staining.

Statistical Analysis: The outcomes were presented as the mean accompanied by the standard error of the mean (SEM). Statistical analysis involved the utilization of analysis of variance (ANOVA). A significance level of $p < 0.05$ was employed as the threshold for determining statistical significance.

Results

Evaluation parameters for callus

The callus cultures were developed with 2% 2,4 D concentrations using leaves explant. Callus index is a parameter

used to access the growth rate of the tissue. Callus index was calculated using following formula [24]:

$$\text{Callus index} = \frac{n * G}{N} * 100$$

where n = total number of callused explants. G = Average weight of callus rating on explant. N = Total number of cultured explants.

The callus index was 54.075 indicating good callus culture growth. The % practical yield for callus extract was 7.45% w/w.

Phytochemical analysis

The liquid chromatography–mass spectrometry (LC–MS) data revealed presence of terpenoids like Asiatic acid, 2-Oxo-5,11(13)-eudesmadien-12,8-olide, Cucurbitacin B, Cucurbitacin E, (+)-cis-5,6-Dihydro-5-hydroxy-4-methoxy-6-(2-phenylethyl)-2H-pyran-2-on, Ganoderic acid C, Asiatic acid, (3 β ,19 α)-3,19,23,24-Tetrahydroxy-12-oleanen-28-oic acid, while flavonoids like Genistin, Allixin, Quercetin, Liquiritic acid on majority. Figures 1 and 2 are the chromatograms of Arjuna callus extract.

Evaluation of ACEN

As per Figs. 3, 4, and 5, the Fourier transform infrared (FTIR) graphs confirmed the compatibility of Arjuna callus extract with polymer PVP K30.

The size of the nanoparticles ranged from 304.4 to 544.1 nm, with a polydispersity index between 0.37 and 0.42. Additionally, the zeta potential ranged from -25.6 to -45.6, indicating that the nanosuspension is stable [25].

The % drug release study revealed that the N4 batch exhibited the lowest release, with $91.58 \pm 0.06\%$ over 35 min. On the other hand, the N9 batch demonstrated the highest % drug release with $99.40 \pm 0.008\%$ over 35 min among all batches. The % drug release data were fitted to standard release equations—zero order, first order, Higuchi model, Hixson, and Kor's peppas. The linear correlation coefficient R square values for all batches are summarized in Table 5. All batches followed first order of kinetics.

Table 7 confirms the optimization of Arjuna callus extract nanosuspension batch.

Stability studies were done for optimized batch N9 as per ICH guideline. Acceleration stability study intermediate storage condition was changed from 30 ± 2 °C and $60 \pm 5\%$ RH, and results are shown in Table 8 and Fig. 15. There was slight increase in particle size from 340 to 347.9 nm on day 90. The % drug content and % drug release were almost unchanged.

Sample Name	AC	Position	P2-E2	Instrument Name	QTOF	User Name	
Inj Vol	3	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	AC.d	ACQ Method	metabolite_ESI_+VE_M	Comment		Acquired Time	4/6/2023 8:10:48 PM

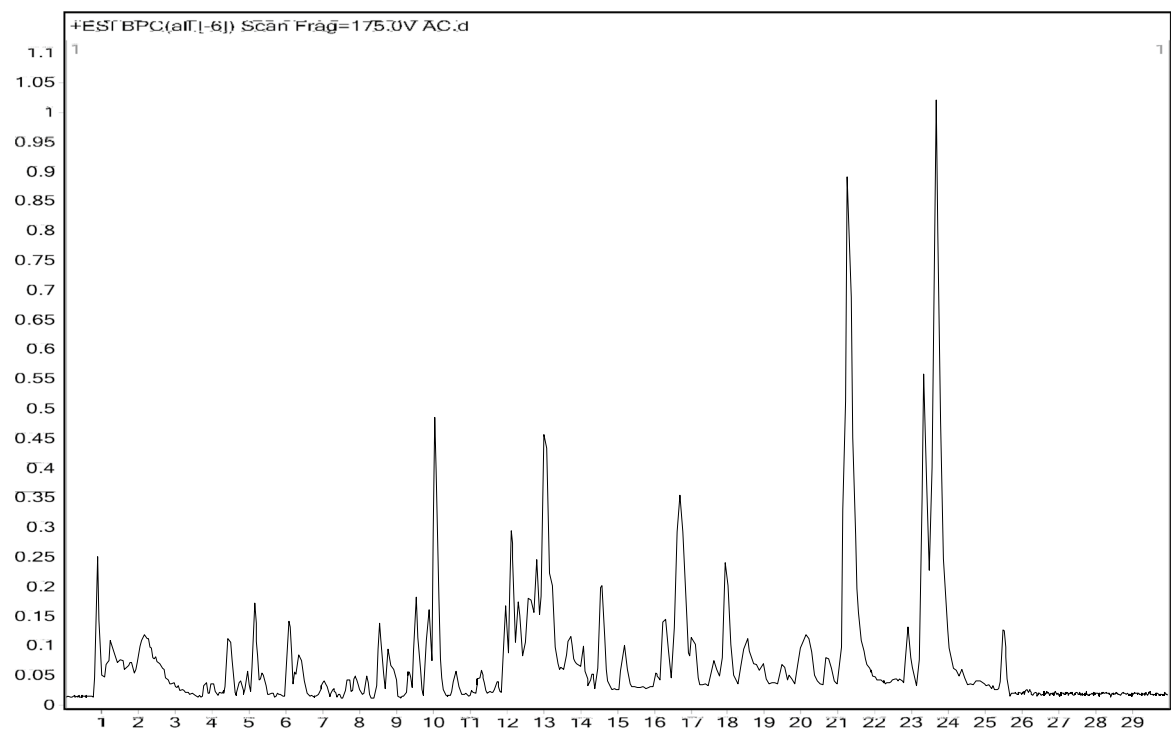


Fig. 1 The chromatogram of callus extract for positively charged compounds

Antilipase assay

The IC50 for standard drug Orlistat was 53.58, while for ACEN was 78.69. The highest % inhibition of pancreatic lipase enzyme for Orlistat was 83.63 at 100 µg/ml, while for ACEN was 78.69 at 100 µg/ml with *p* value < 0.0001.

Antioxidant activity

The IC50 value for the standard drug sample, ascorbic acid, was 137.57, while for ACEN, it was 42.49. Remarkably, ACEN exhibited a percentage inhibition surpassing that of ascorbic acid, and this trend was observed to intensify with higher concentrations. The *p*-value of 0.0009 represents the statistical significance of these results [26].

Animal study

According to Fig. 18, the body weight of rats increased in all groups throughout the experiment, except for the normal group. The control group exhibited the most significant increase in body weight on the 15th and 30th days when compared to the normal group. The body weights of the standard and ACEN-treated groups were lower than the control group, but were similar to the standard group.

Figure 19 shows the lipid profile of all groups. The control group exhibited an increase in total cholesterol (TC) levels compared to the normal group. However, there was a slight decrease in TC levels in both the ACEN-treated and standard groups when compared to the control group. The control group showed a decrease

Sample Name	AB	Position	P2-E1	Instrument Name	QTOF	User Name	
Inj Vol	3	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	AB-ve.d	ACQ Method	metabolite_ESI_-VE_M	Comment		Acquired Time	4/7/2023 3:51:59 AM

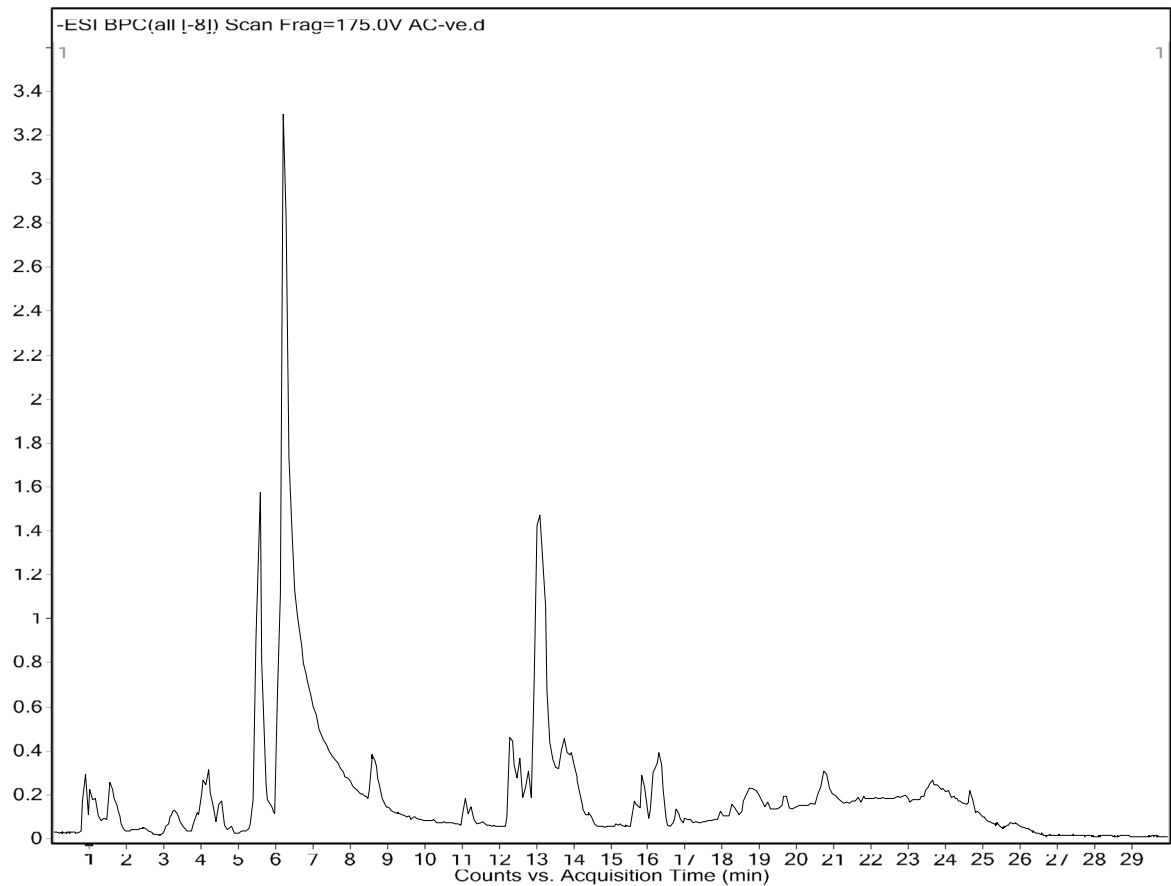


Fig. 2 Chromatogram of callus extract for negatively charged compounds

in TG levels, while the ACEN-treated and standard groups maintained stable levels, similar to the normal group. The control group had lower levels of HDL, while the ACEN-treated group had slightly higher levels. Low-density lipoprotein (LDL) level was increased in the control group but was maintained in ACEN-treated group as that of normal. Very low-density lipoprotein (VLDL) levels were elevated in the control group but remained relatively stable in the ACEN-treated and standard groups, approaching levels similar to the normal group. The data underwent ANOVA, and it

confirmed its statistical significance with a *p*-value of less than 0.0001.

Atherogenic index (AIS)

The AIS for the ACEN group was less than the control group and almost similar to that of standard group. In a study involving rats that fed with HFD diet for 30 days, the AIS increased from 0.373278 in the normal group to 1.48455 in the control group (*p* < 0.0001). However, in the ACEN group, the AIS was 1.09205, which was like the standard group, with a value of 1.0563.

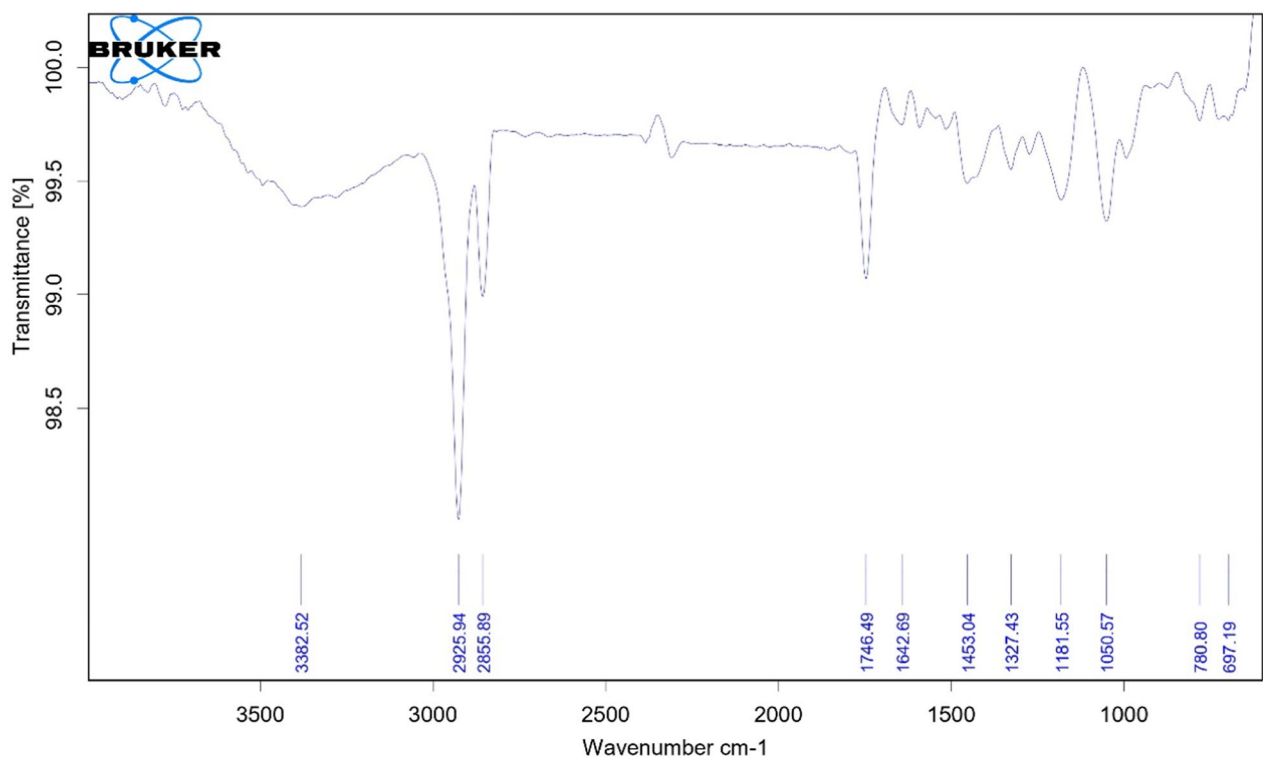


Fig. 3 FTIR of callus extract

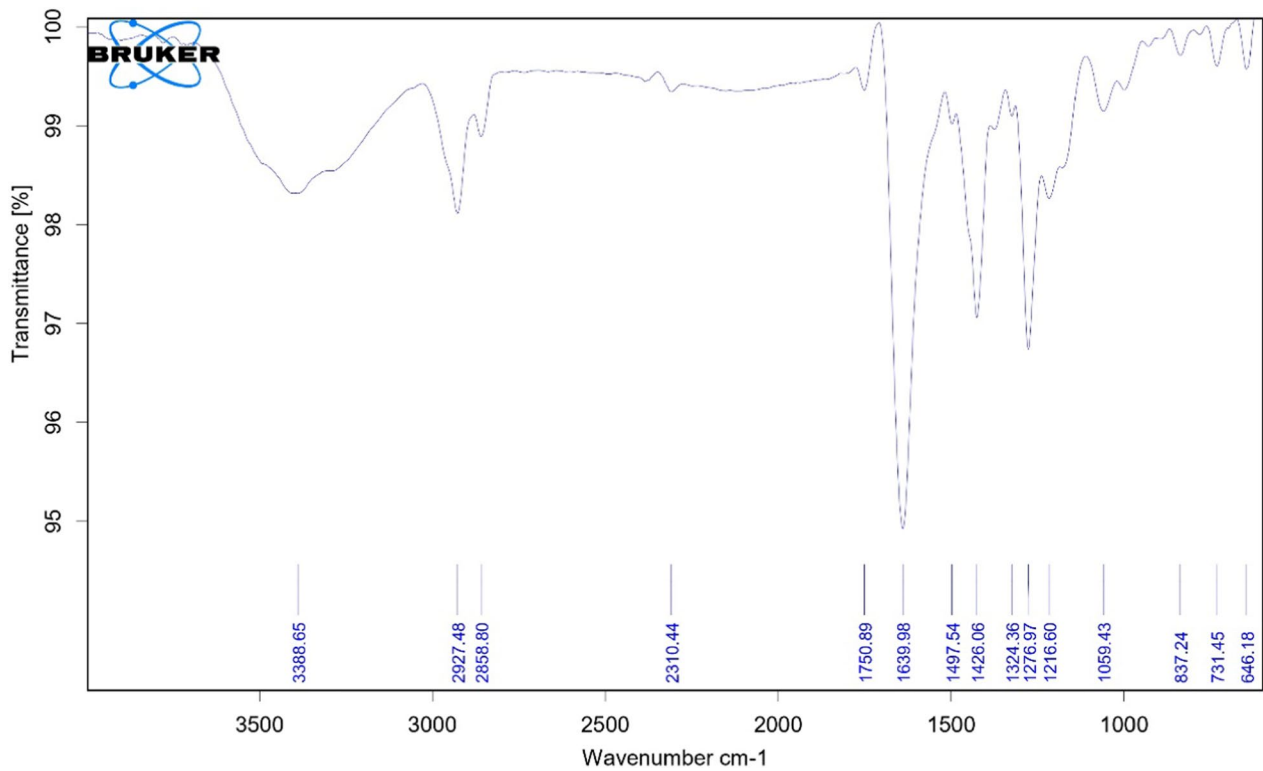


Fig. 4 FTIR of physical mixture of callus extract and PVP K30

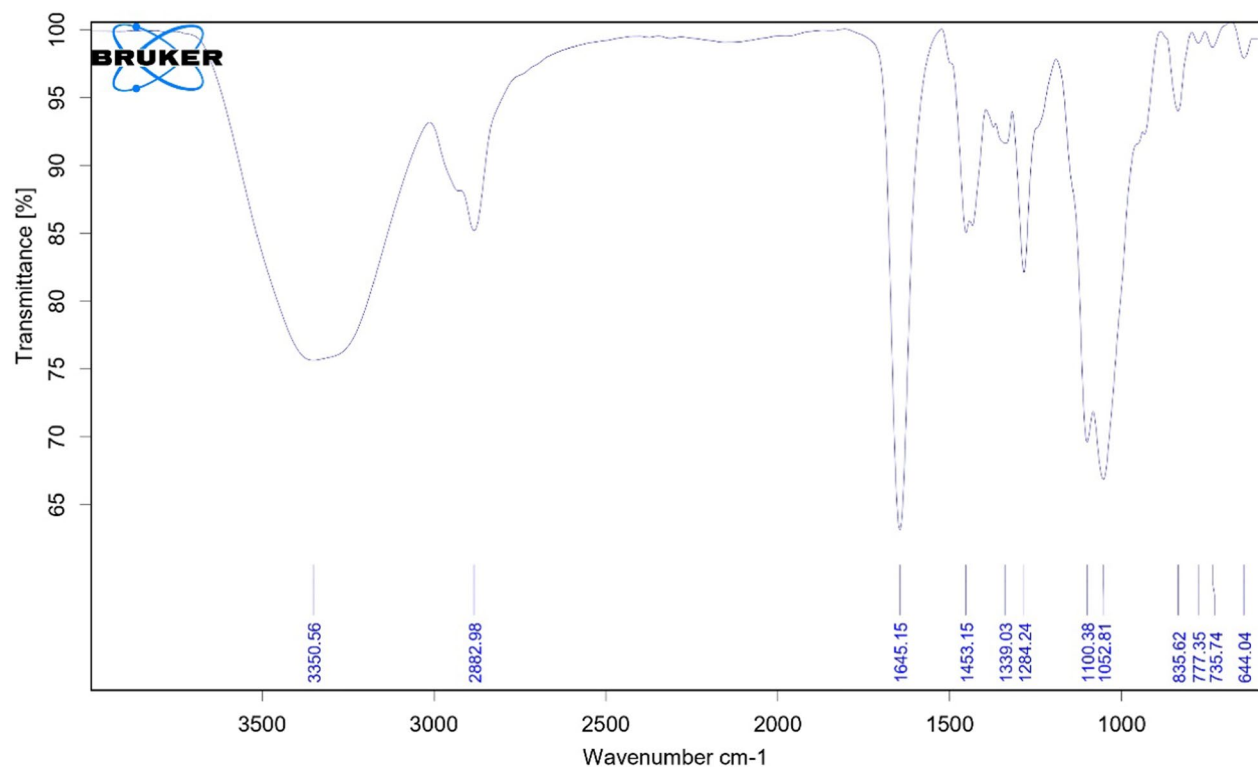


Fig. 5 FTIR of nanosuspension of callus extract

Histology of the aorta

In the control group, there was a noticeable narrowing of the aortic lumen due to lipid deposition. Conversely, in rats treated with ACEN, the aortic lumen appeared almost identical to that of the normal group. Additionally, the standard group’s rats exhibited more significant atherosclerosis-related narrowing of the aortic lumen compared to the ACEN-treated group (Figs. 21, 22, 23, 24).

Discussion

Phytochemical analysis

The callus cultures are known to exhibit the phytoconstituents that are not present in mother plants naturally [27]. The LC–MS data showed the presence of terpenoids like (3beta, 19alpha)-3,19,23,24-tetrahydroxy-12-oleanen-28-oic acid, 3-[4-Hydroxy-3-(3-methyl-2-butenyl)phenyl]-2-propenal which are absent in Arjun tree naturally [8] (Table 1).

Table 1 Phytoconstituents of callus and their pharmacological activity

Name of compound	Pharmacological activity
4-Hydroxy-L-threonine	It plays a crucial role in facilitating healthy fat metabolism within the liver, supporting the smooth functioning of the digestive and intestinal systems, and promoting efficient metabolic processes and assimilation [38]
Allixin	Antioxidant, antiatherogenic, anticancer activity [39, 40]
Genistin	Antioxidant, antiadipogenic and antilipogenic agent [41]
Ellagic acid	Antioxidant, anti-inflammatory, antimutagenic, antiproliferative, antiallergic, antiatherosclerotic, cardioprotective, hepatoprotective, nephroprotective, and neuroprotective property [31, 42]
Quercetin	Reduces oxidative stress, inhibits low-density lipoproteins oxidation and platelet aggregation, and acts as vasodilator in blood vessels [43]
Calophyllum B	Anti-inflammatory activity [44]

Table 1 (continued)

Name of compound	Pharmacological activity
Candoxatrilat	Endopeptidase inhibitor, useful in congestive heart failure [45]
(R)-Heraclenol	Inhibits histidine biosynthesis selectively and shows antibacterial activity [46]
2-Oxo-5,11(13)-eudesmadien-12,8-olide	Anti-inflammatory, antiparasitic, antiviral, and cytotoxic activity [47]
Austrobailignan 7	Anticancer activity [48]
Schleicherastatin 6	Anticancer activity [49]
Mitoxantrone	Anticancer, anti-inflammatory activity and inhibits IFN- γ , TNF- α , and IL-2 [50, 51]
Paeonilactone C	Neuroprotective and antioxidant activity [52, 53]
Teasterone	Anti-inflammatory activity [54]
(5 α ,6 α)-5,6-Epoxyergosta-7,22-dien-3-ol	Antioxidant and anti-inflammatory activity [55]
Convallasaponin	Tumor-specific cytotoxic activity [56]
Campesteryl ferulate	Antioxidant activity, hypolipidemic effect, elevation in HDL cholesterol level, suppression of platelet aggregation, and inhibition of tumor promotion [32]
Smilagenone	In cognitive impairment treatment [57]
(25R)-5 β -spirostan-3 β -ol	Lowers bold cholesterol level [58]
Cucurbitacin B	Anti-inflammatory, antioxidant, in treatment of neurodegenerative diseases, diabetes mellitus, and cancers, decreases lipid accumulation and antiatherosclerotic [33, 34]
Euphornin	In cancer treatment [59]
Pyropheophorbide a	In cancer treatment [60]
Cucurbitacin E	Anti-inflammatory, antiangiogenic, immunomodulatory, cytotoxic, cytostatic, and hepatoprotective property [61]
Ciclesonide	In treatment of asthma [62]
Methyl 3 β -hydroxy-13(18)-oleanen-28-oate	Antiosteoporotic activity [63]
Schleicherastatin 6	In treatment of cancer [64]
1-O-Galloylpedunculagin	Antioxidant activity [65, 66]
(+)-cis-5,6-Dihydro-5-hydroxy-4-methoxy-6-(2-phenylethyl)-2H-pyran-2-one	A triterpenoid, pharmacological activity not known yet
Ellagic acid	It affects lipid metabolism by promoting cholesterol efflux and decreasing LDL uptake, while also possessing antioxidant, liver-protective, antisteatotic, anticholestatic, antifibrogenic, antihepatocarcinogenic, and antiviral activity [31]
Ganoderic acid C	Anti-inflammatory (inhibition of TNF α) activity [35, 36, 67]
Ceanothic acid	Anticancer and antimicrobial activity [68, 69]
Asiatic acid	Antioxidant, anti-inflammatory and antihyperlipidemia activity [37, 70]
Azukisapogenol	Antibacterial and antifungal activity [71]
Liquiritic acid	Atheroprotective, anti-inflammatory activity [72, 73]
(3 β ,19 α)-3,19,23,24-Tetrahydroxy-12-oleanen-28-oic acid	A terpenoid showing similar chemical structure to arjunic acid and arjunin acid whose pharmacological activity is not known yet

Evaluation of ACEN

Considering Fig. 3, 4, and 5, the alkyn –OH stretching at 3300, alkyl C–H stretch at 2925 and 2855, C=O stretch at 1746 and 1642, C–O at 1000–1300 were preserved in the mixture IR spectra, indicating the compatibility of the callus extract with PVP K 30 (Table 2).

Considering two independent factors concentration of polymer PVPK30 (1, 1.5, and 2%) and mixing speed (1000, 1500, and 2000) at three levels each, 9 batches were analyzed using design of experiment software version 6 (Fig. 6). The analysis employed a linear model (Table 3) to examine the correlation between polymer concentration

Table 2 Batches of nanosuspension

Batch	Concentration PVPK30 (% w/v)	Mixing speed (rpm)
N1	1	1000
N2	1.5	1000
N3	2	1000
N4	1	1500
N5	1.5	1500
N6	2	1500
N7	1	2000
N8	1.5	2000
N9	2	2000

Select	Std	Run	Factor 1 A:Polymer c... %	Factor 2 B:Speed of r... rpm	Response 1 Particle size nm	Response 2 PI	Response 3 Zeta potential meV	Response 4 %Drug content %	Response 5 % Drug entr... %
5		1	1.5	1500	377	0.38	-33.2	97.67	96.59
	7	2	1	2000	403.1	0.41	-38.5	96.2	95.72
	8	3	1.5	2000	304.4	0.41	-25.6	97.34	96.64
	3	4	2	1000	526.6	0.4	-38	98.22	95.13
	9	5	2	2000	382.9	0.39	-32.1	99.69	98.92
	1	6	1	1000	481	0.42	-42	99.75	98.35
	6	7	2	1500	372	0.37	-45.6	98.52	96.12
	2	8	1.5	1000	544.1	0.41	-29.1	98.82	94.68
	4	9	1	1500	507.47	0.4	-43.2	97.32	95.99

Fig. 6 Summary of design of experiment software analysis

Table 3 ANOVA table for particle size of nanosuspension

ANOVA for Response Surface (Linear + User Added Terms) model (Aliased)						
Analysis of variance table [Partial sum of squares—Type III]						
Source	Sum of squares	df	Mean square	F value	p-value Prob > F	
Model	37,485.52	2	18,742.76	6.16	0.0352	Significant
A-Polymer concentration	2019.23	1	2019.23	0.66	0.4465	
B-Speed of rotation	35,466.28	1	35,466.28	11.65	0.0143	
ACERTUV	0.000	0				
Residual	18,267.14	6	3044.52			
Cor total	55,752.66	8				

Table 4 Effect of polymer concentration and speed of mixing on particle size

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	433.17	1	18.39	388.17	478.18	
A-Polymer concentration	−18.35	1	22.53	−73.46	36.77	1.00
B-Speed of rotation	−76.88	1	22.53	−132.00	−21.76	1.00
ACERTUV	= ACERTUV + A					

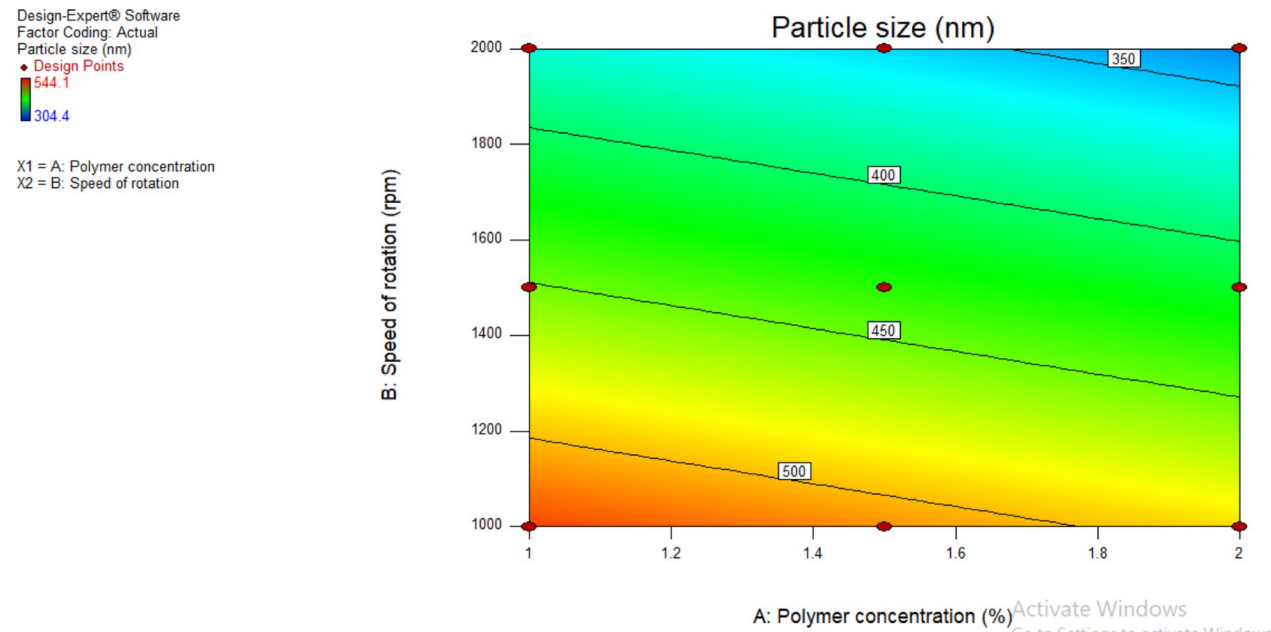


Fig. 7 2D contour plot for particle size analysis of nanosuspension batches

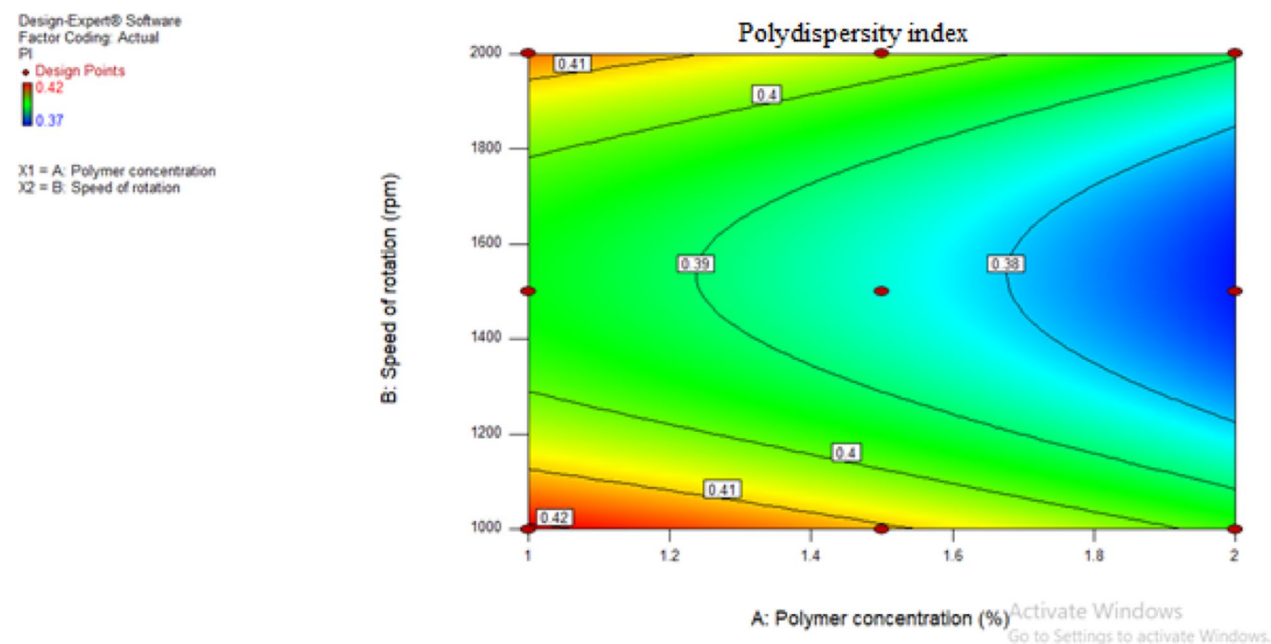


Fig. 8 2D contour plot for polydispersity index analysis of nanosuspension batches

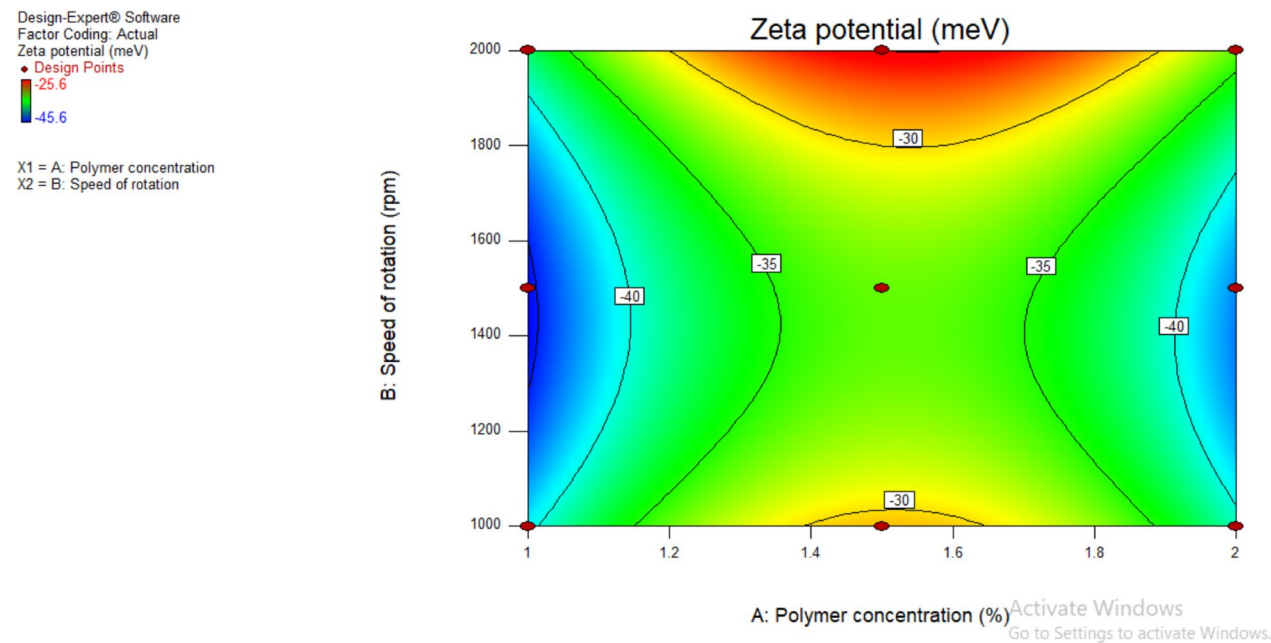


Fig. 9 2D contour plot for zeta potential analysis of nanosuspension batches

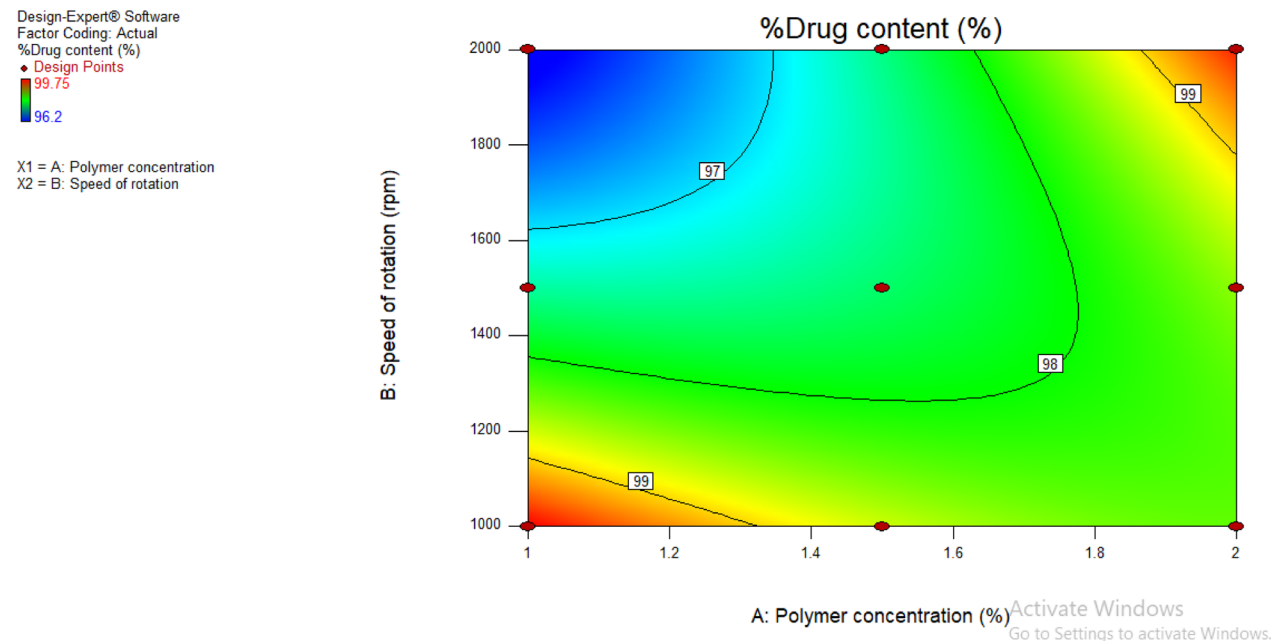


Fig. 10 2D contour plot for % drug content analysis of nanosuspension batches

and mixing speed regarding particle size of ACEN. We confirmed the significance of the data input with an *F*-value of 6.16. In Table 4, we observed a negative coefficient for both polymer concentration and mechanical

stirring speed. A positive sign denotes a synergistic effect, while a negative sign indicates an antagonistic effect. The interaction plots (Figs. 7, 8, 9, 10, 11) illustrated that the relative slopes of factors—particle size, polydispersity



We found that all batches N1 to N9 followed first-order kinetics (Table 5). As per Fig. 12, we found that the

The analysis of variance (ANOVA) identified 10 potential solutions based on the criteria outlined in Table 6. The combination of 2% PVP K30 and a mechanical stirring speed of 2000 rpm scored 0.917 desirability. The batch optimization is evident from Table 7. The transmission electron microscopy image displayed the nanoscale dimensions and encapsulation of Arjuna callus extract within a polymer matrix (Figs. 13, 14).

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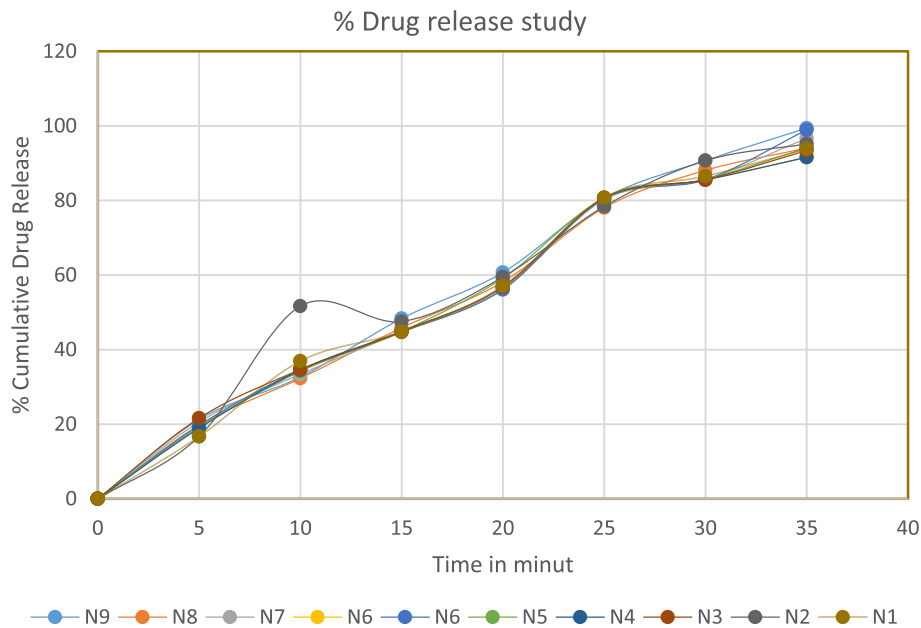


Fig. 12 % Drug release study

Table 6 Constrains for optimization of nanosuspension

Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
A:Polymer concentra- tion	Is in range	1	2	1	1	3
B:Speed of rotation	Is in range	1000	2000	1	1	3
Particle size	Minimize	304.4	544.1	1	1	4
PI	Is in range	0.37	0.42	1	1	3
Zeta potential	Is in range	-45.6	-25.6	1	1	3
%Drug content	Maximize	96.2	99.75	1	1	4
% Drug entrapment efficiency	Maximize	94.68	98.92	1	1	4

Table 7 Comparison of theoretical value and practical value of optimized nanosuspension

Parameter	Particle size (nm)	Polydispersity index (PI)	Zeta potential	% Drug content	% Drug entrapment efficiency
ANOVA suggested theo- retical value	337.946	0.391	−33.667	99.638	98.603
Practical value	340.9	0.399	−34.1	99.83607	98.91557

The N9 sample did not change physical appearance throughout the acceleration stability study. As per results discussed in Table 8 and Fig. 15, we found that there was slight increase in the size of nanoparticle, but it was within acceptable limit. The slight increase in particle size of ACEN did not affect the % drug content of % drug release.

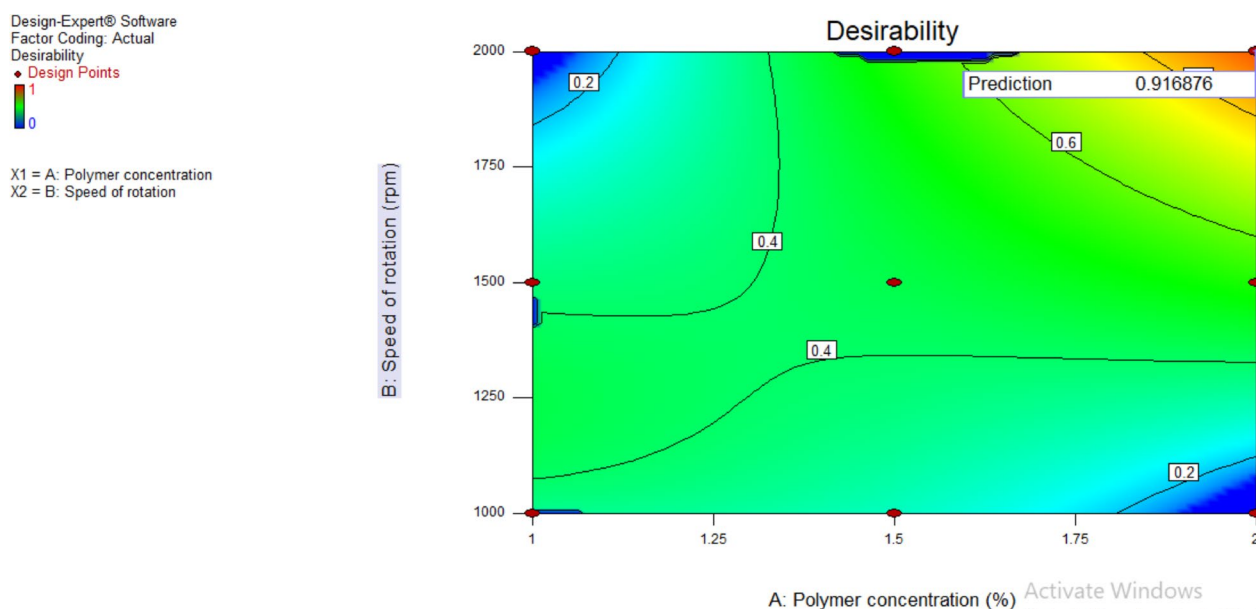


Fig. 13 2D contour plot for desirability for nanosuspension batch optimization

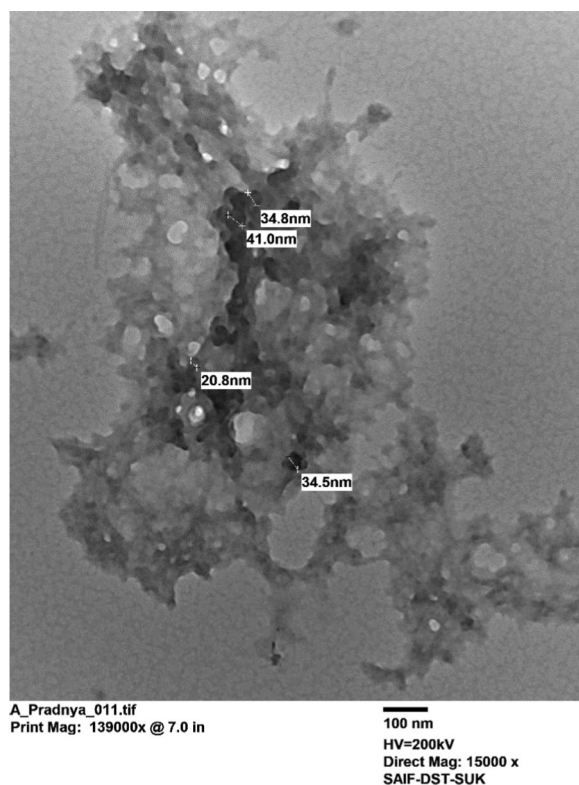


Fig. 14 Transmission electron microscope image of ACEN

Antilipase assay

Hyperlipidemia exhibits strong associations with various metabolic conditions, including diabetes, atherosclerosis, and hypertension. Within the duodenum, pancreatic lipase plays a pivotal role in the digestion and absorption of lipids, breaking down triacylglycerols into monoacylglycerols and fatty acids. The pancreatic lipase inhibitors have a potential to induce hypolipidemic effects, offering a promising atherosclerosis management [28, 29]. We found that the % inhibition of lipase enzyme by ACEN was concentration dependent (Fig. 16). The antilipase activity of ACEN might be due to presence of 4-Hydroxy-L-threonine, Genistin, Cucurbitacin B, Ganoderic acid C, Asiatic acid, Ellagic acid, Campesteryl ferulate. This could be one of the antiatherosclerotic mechanisms of ACEN.

Antioxidant activity

Antioxidants play a pivotal role in the treatment of atherosclerosis through a range of mechanisms. These mechanisms encompass the inhibition of LDL oxidation, the reduction production of reactive oxygen species, the suppression of cytokine secretion, the prevention of atherosclerotic plaque formation, prevention

Table 8 Acceleration stability studies for N9 batch

Period	Particle size (nm)	PDI	% Drug content
15 days	341.5	0.399	99.66 ± 0.3
30 days	341.7	0.399	99.63 ± 0.4
60 days	345.1	0.340	99.60 ± 0.7
90 days	347.9	0.340	99.57 ± 0.1

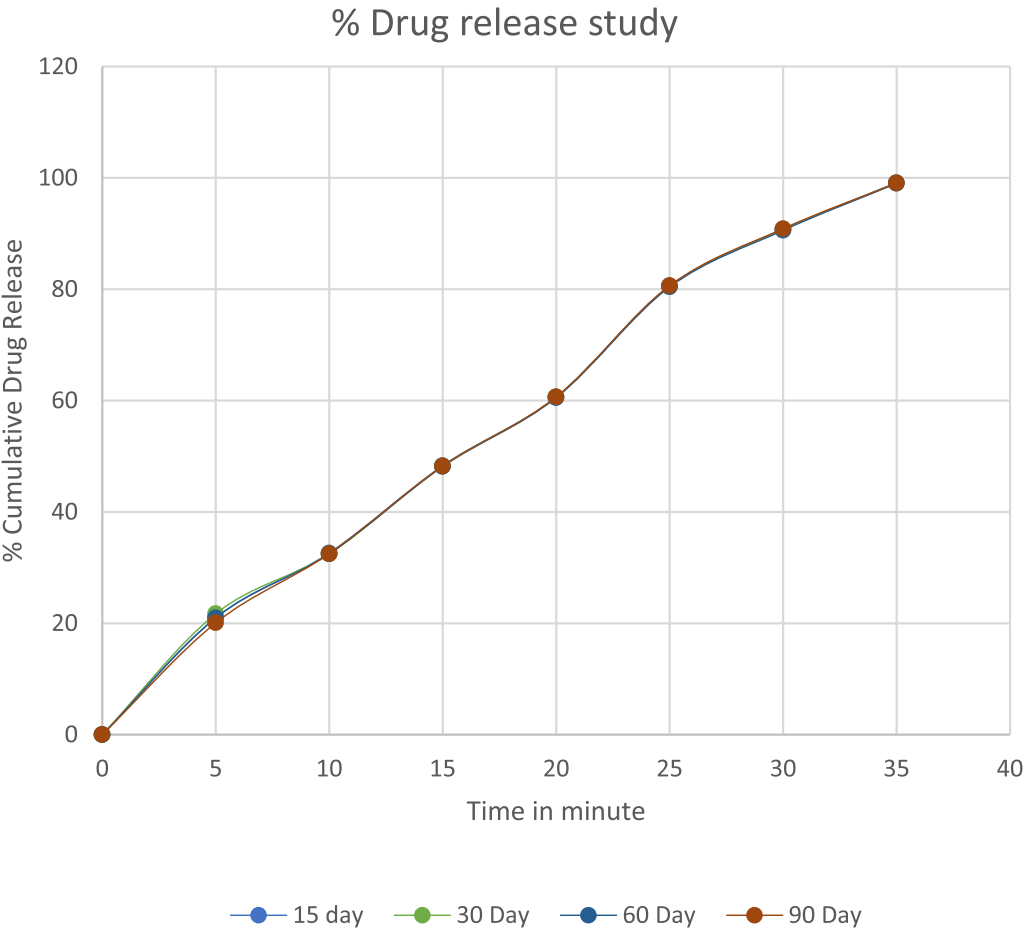


Fig. 15 % Drug release studies of N9 batch for acceleration stability studies

of platelet aggregation, the hindrance of mononuclear cell infiltration, the enhancement of endothelial function and vasodilation, the promotion of nitric oxide (NO) bioavailability, the modulation of adhesion molecule expression (e.g., VCAM-1 and ICAM-1) on endothelial cells, and the mitigation of foam cell formation. The effectiveness of these diverse antioxidant actions remains an open question, but it appears that a multi-antioxidant approach may offer a more

effective strategy for antioxidant therapy. We found the ACEN shows better antioxidant activity than standard drug ascorbic acid (Fig. 17). The presence of various compounds, including Allixin, Genistin, Ellagic acid, Quercetin, Paeonilactone C, (5x,6x)-5,6-Epoxyergosta-7,22-dien-3-ol, Campesteryl ferulate, and others, is likely responsible for the observed antioxidant activity of ACEN [19, 30].

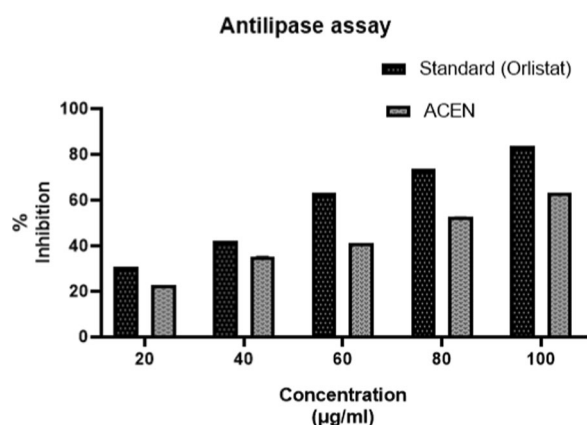


Fig. 16 Antilipase assay for ACEN

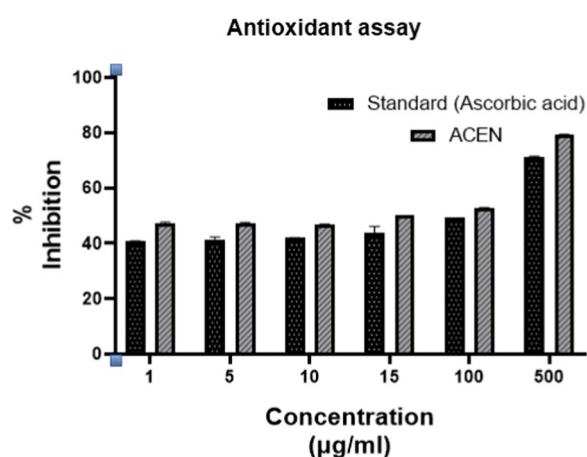


Fig. 17 Antioxidant activity assay of ACEN

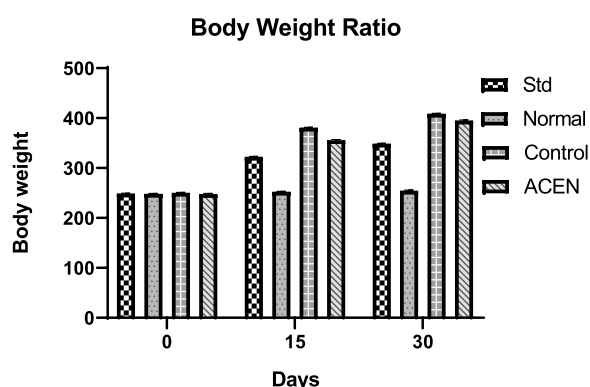


Fig. 18 Summary of body weight of animal groups at 0, 15, and 30 days

Animal study

Figure 18 summarizes the body weight of animals during the study (Fig. 18). We discovered that ACEN was more effective than the standard drug atorvastatin at decreasing TC, TG, HDL, LDL, and VLDL (Fig. 19). Ellagic acid plays a role in lipid metabolism by promoting cholesterol efflux and reducing LDL uptake. The terpenoids, such as Ellagic acid and Asiatic acid, are known for their potential in managing hyperlipidemia. The potential cause of ACEN's lipid-lowering effect could be attributed to the presence of these terpenoids. [31–37].

Atherogenic index of serum

The AIS is a measure of the potential risk of developing atherosclerosis. When the AIS is higher, there is a greater potential for atherosclerosis and an increased likelihood of cardiovascular disease. It is important to note that the AIS is influenced by the levels of HDL. When HDL levels are higher, the atherogenic index is lower, reducing the risk of atherosclerosis [23]. From Fig. 20, we found that the AIS of ACEN (1.05 ± 0.035) was slightly lesser than the AIS of standard drug atorvastatin (1.06 ± 0.053) emplacing the antiatherosclerotic potential of ACEN (Figs. 21, 22, 23, 24).

Histology of the aorta

The histopathological results confirmed the antiatherosclerotic potential of ACEN. The presence of phytoconstituents in ACEN like Ellagic acid, Quercetin, Calophyllum B, (25R)-5beta-spirostan-3beta ol Liquiritic acid known for their lipid-lowering, antioxidant, and anti-inflammatory properties, may be responsible for its antiatherosclerotic effect. The histopathological analysis demonstrated that nanotherapeutics exhibited superior efficacy when compared to the standard drug Atorvastatin, highlighting the enhanced effectiveness of the callus extract attributed to its nanoformulation. Altogether, the comprehensive study has affirmed ACEN's potential in atherosclerosis treatment.

Conclusion

A thorough analysis of phytochemical composition of the callus extract predominantly revealed the presence of flavonoids and terpenoids, endowed with antioxidant, hypolipidemic, and anti-inflammatory activity. The callus culture extract was then transformed into a nanosuspension to achieve better efficacy of the medicament. The optimized nanosuspension formulation was subjected

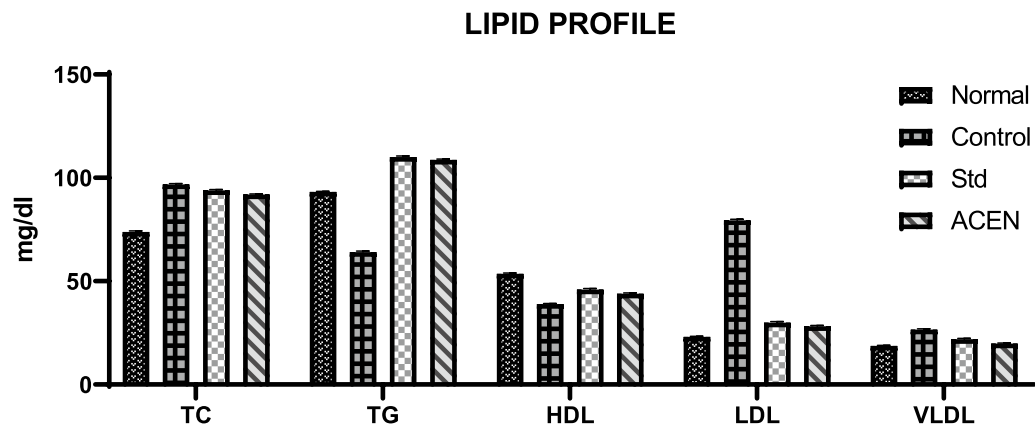


Fig. 19 Summary lipid profile of animal groups

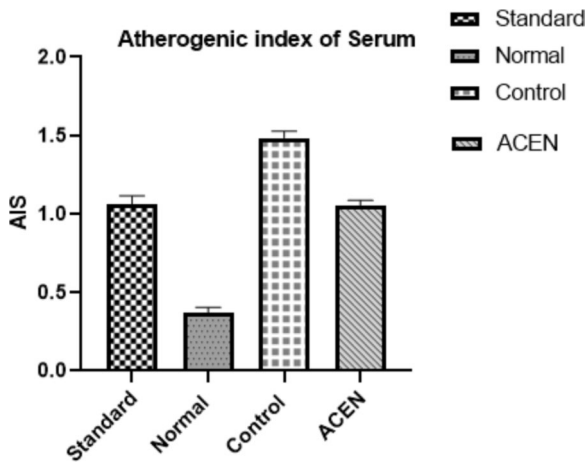


Fig. 20 Summary atherogenic index of serum

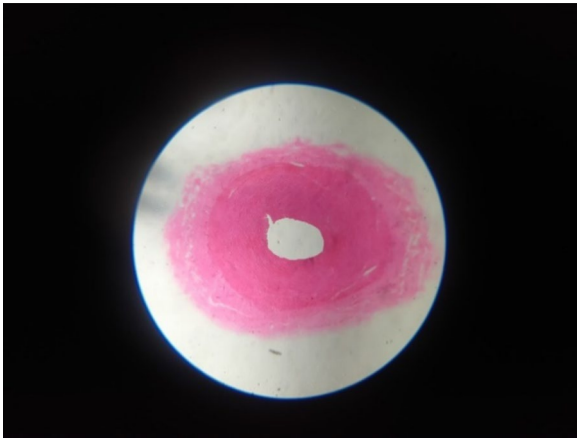


Fig. 22 Rat aorta in normal group

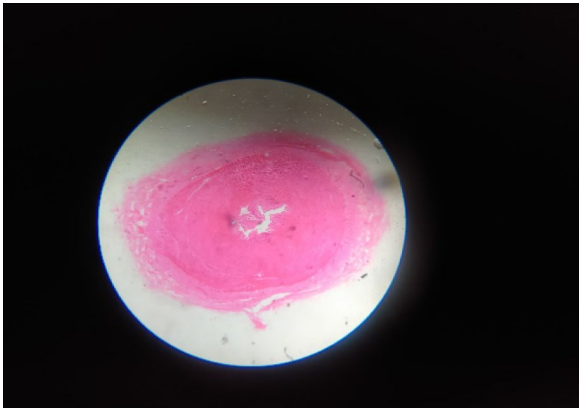


Fig. 21 Rat aorta in control group

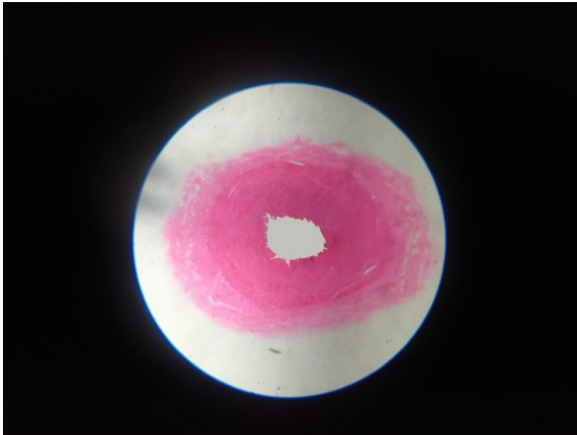


Fig. 23 Rat aorta in ACEN group

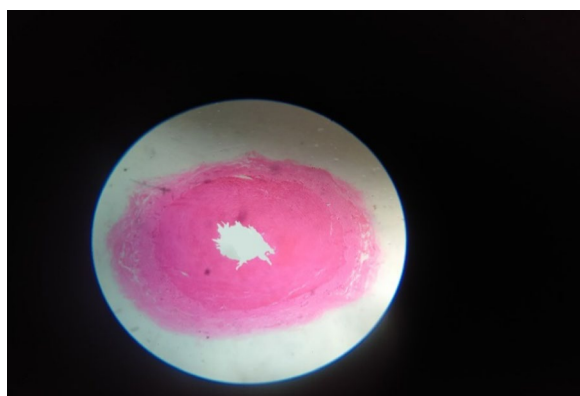


Fig. 24 Rat aorta in standard group

to comprehensive in vitro and in vivo studies, aimed to confirm its antiatherosclerotic potential. The study findings suggest that ACEN has the potential to be a natural-origin drug for the treatment of atherosclerosis.

Abbreviations

ACEN	Arjuna callus extract nanoparticles
LC-MS	Liquid chromatography–mass spectrometry
PVP K30	Polyvinylpyrrolidone K30
RH	Relative humidity
PPL	Porcine pancreatic lipase
p-NPB	P-nitrophenyl butyrate
DPPH	1,1-Diphenyl-2, picryl-hydrazyl
TC	Total cholesterol
TG	Triglyceride
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
VLDL	Very low-density lipoprotein
FTIR	Fourier transform infrared

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by PPW. The first draft of the manuscript was written by PPW, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data for graphs 12 to 16 are available on request.

Declarations

Ethics approval

This study was performed in line with the principles of laid down by Institute Animal Ethics Committee. Approval was granted by the Ethics Committee of

Appasaheb Birnale College of Pharmacy, Sangli (Date 20/01/2022 /No IAEC/ ABCP/13/2021–22).

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Human and animal rights

Not applicable.

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References

1. Nakhband A, Eskandani M, Omid Y, Saeedi N, Ghaffari S, Barar J, Garjani A (2018) Combating atherosclerosis with targeted nanomedicines: recent advances and future prospective. *BiolImpacts*: BI 8(1):59
2. Perera B, Wu Y, Nguyen NT, Ta HT (2023) Advances in drug delivery to atherosclerosis: investigating the efficiency of different nanomaterials employed for different type of drugs. *Materials Today Bio* 100767
3. Schade DS, Duro T, Eaton RP (2023) Optimal prescribing of statins to reduce cardiovascular disease. *Am J Med*. <https://doi.org/10.1016/j.jmbio.2023.100767>
4. Subramaniam S, Subramaniam R, Rajapandian S, Uthrapathi S, Gnana-manickam VR, Dubey GP (2011) Anti-atherogenic activity of ethanolic fraction of Terminalia arjuna bark on hypercholesterolemic rabbits. *Evidence-Based Complementary and Alternative Medicine*, 2011; <https://pubmed.ncbi.nlm.nih.gov/21785628/>
5. Hosseini A, Sahebkar A (2017) Reversal of doxorubicin-induced cardiotoxicity by using phytotherapy: a review. *J Pharmacopuncture* 20(4):243
6. Dwivedi S, Chopra D (2014) Revisiting Terminalia arjuna—an ancient cardiovascular drug. *J Tradit Comp Med* 4(4):224–231
7. Eggadi V, Korupozu SC, Kumar Korupolu B, Sheshagiri SB, Kumar S, Jupally VR (2014) Evaluation of protective effect of different doses of terminalia arjuna bark ethanolic extract on cisplatin induced oxidative nephrotoxicity in rats. *Iraqi J Pharm Sci (P-ISSN 1683-3597 E-ISSN 2521-3512)*, 23(2):89–98
8. Kapoor D, Vijayvergiya R, Dhawan V (2014) Terminalia arjuna in coronary artery disease: ethnopharmacology, pre-clinical, clinical and safety evaluation. *J Ethnopharmacol* 155(2):1029–1045
9. Kumari V, Sangal A (2020) Antimicrobial study of Arjuna Terminalia loaded PLGA nanoparticle. *Indian J Biochem Biophys* 57(3):291–297. <https://nopr.niscpr.res.in/bitstream/123456789/54469/1/IJBB%2057%283%29%20291-297.pdf>
10. Pandey AK, Mandal AK (2012) Sustainable Harvesting of Terminalia arjuna (Roxb.) Wight & Arnot (Arjuna) and Litsea glutinosa (Lour.) Robinson (Maida) Bark in Central India. *J Sustain Forestry* 31(3):294–309
11. Thomas A, Radhakrishnan EK (2023) Acute and sub-acute oral toxicity studies of hydroalcoholic extract of Terminalia arjuna (Roxb.) Bark in Rodents. *Trop J Natural Prod Res* 7(7)
12. Lewis DR, Petersen LK, York AW, Ahuja S, Chae H, Joseph LB, Moghe PV (2016) Nanotherapeutics for inhibition of atherogenesis and modulation of inflammation in atherosclerotic plaques. *Cardiovasc Res* 109(2):283–293; <https://doi.org/10.1093/cvr/cwv237>
13. Rai VK, Gupta GD, Potttoo FH, Barkat MA (2020) Potential of nano-structured drug delivery system for phytochemistry delivery. *Nanophytomed: Concept Clin* 89–111
14. Chakraborty K, Shivakumar A, Ramachandran S (2016) Nano-technology in herbal medicines: a review. *Int J Herb Med* 4(3):21–27
15. Sawant KK, Patel MH, Patel K (2016) Cefdinir nanosuspension for improved oral bioavailability by media milling technique: formulation,

- characterization and in vitro–in vivo evaluations. *Drug Dev Ind Pharm* 42(5):758–768
16. Ramesh Y, Sarayu B, Chandana GH, Neelima O, Sana S (2021) Formulation and evaluation of lamivudine nanosuspension. *J Drug Deliv Therap* 11(4-5):71–77
17. Roh C, Jung U (2012) Screening of crude plant extracts with anti-obesity activity. *Int J Mol Sci* 13(2):1710–1719
18. Raut DG, Bhosale RB, Lawand AS, Hublikar MG, Kadu VD, Patil SB, Choudhari PB (2022) Syntheses, molecular docking and biological evaluation of 2-(2-hydrazinyl) thiazoles as potential antioxidant, anti-inflammatory and significant anticancer agents. *Recent Adv Inflam Allergy Drug Discov* 16(2):96–106
19. Cherubini A, Vigna GB, Zuliani G, Ruggiero C, Senin U, Fellin R (2005) Role of anti-oxidants in atherosclerosis: epidemiological and clinical update. *Curr Pharm Des* 11(16):2017–2032
20. Biplav S, Sindhura G, Shivalinge GK (2018) To evaluate the anti-atherosclerotic potential of quercetin in alloxan-induced diabetic rats fed with high-fat diet. *Asian J Pharm Clin Res* 11(3):379–383
21. Machhi JP, Shah NN (2012) Study of antiatherosclerotic activity of polyherbal preparation using rat as an experimental animal model. *Int J Pharm Sci Res* 3(10):4010
22. Zhang J, Almoallim HS, Alharbi SA, Yang B (2021) Anti-atherosclerotic activity of Betulinic acid loaded polyvinyl alcohol/methylacrylate grafted Lignin polymer in high fat diet induced atherosclerosis model rats. *Arab J Chem* 14(2):102934
23. Sa'adah NN, Purwani KI, Nurhayati APD, Ashuri NM (2017) Analysis of lipid profile and atherogenic index in hyperlipidemic rat (*Rattus norvegicus* Berkenhout, 1769) that given the methanolic extract of *Parijoto* (*Medinilla speciosa*). In: AIP Conference Proceedings (Vol. 1854, No. 1). AIP Publishing
24. Wakhlu AK, Barna KS (1989) Callus initiation, growth and plant regeneration in *Plantago ovata* Forsk. cv. GI-2. *Plant Cell Tissue Organ Cult* 17:235–241
25. Rezaei A, Abdollahi H, Derikvand Z, Hemmati-Sarapardeh A, Mosavi A, Nabipour N (2020) Insights into the effects of pore size distribution on the flowing behavior of carbonate rocks: linking a nano-based enhanced oil recovery method to rock typing. *Nanomaterials* 10(5):972
26. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM (2022) Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules* 27(4):1326
27. Malarz J, Stojakowska A, Szneler E, Kisiel W (2005) Furofuran lignans from a callus culture of *Cichorium intybus*. *Plant Cell Rep* 24:246–249
28. Chang Y, Zhang D, Yang G, Zheng Y, Guo L (2021) Screening of anti-lipase components of *Artemisia argyi* leaves based on spectrum-effect relationships and HPLC-MS/MS. *Front Pharmacol* 12:675396
29. Tsutsumi K (2003) Lipoprotein lipase and atherosclerosis. *Curr Vasc Pharmacol* 1(1):11–17
30. Malekmohammad K, Sewell RD, Rafieian-Kopaei M (2019) Antioxidants and atherosclerosis: mechanistic aspects. *Biomolecules* 9(8):301
31. García-Niño WR, Zazueta C (2015) Ellagic acid: pharmacological activities and molecular mechanisms involved in liver protection. *Pharmacol Res* 97:84–103. <https://doi.org/10.1016/j.phrs.2015.04.008>
32. Srikaeo K (2014) Organic rice bran oils in health. Wheat and rice in disease prevention and health, 453–465
33. Dai S, Wang C, Zhao X, Ma C, Fu K, Liu Y, Li Y (2022) Cucurbitacin B: a review of its pharmacology, toxicity, and pharmacokinetics. *Pharmacol Res* 106:587
34. Park SY, Kang HM, Oh JW, Choi YW, Park G (2022) Cucurbitacin B-, E-, and I-Induced Browning of White Adipocytes Is Promoted by the Inhibition of Phospholipase D2. *Int J Mol Sci* 23(23):15362. <https://doi.org/10.3390/ijms232315362>
35. Liang C, Tian D, Liu Y, Li H, Zhu J, Li M, Xia J (2019) Review of the molecular mechanisms of *Ganoderma lucidum* triterpenoids: Ganoderic acids A, C2, D, F, DM, X and Y. *Eur J Med Chem* 174:130–141. <https://doi.org/10.1016/j.ejmech.2019.04.039>
36. Liu C, Yang N, Folder W, Cohn J, Wang R, Li X (2012) Ganoderic acid C isolated from *Ganoderma lucidum* suppress Lps-induced macrophage Tnf- α production by down-regulating Mapk, NF-kappab and Ap-1 signaling pathways. *J Allergy Clin Immunol* 129(2):127
37. Fong LY, Ng CT, Cheok ZL, Moklas MAM, Hakim MN, Ahmad Z (2016) Barrier protective effect of asiatic acid in TNF- α -induced activation of human aortic endothelial cells. *Phytomedicine* 23(2):191–199
38. PubChem. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 6288, Threonine; [cited 2023 Oct. 8]. Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/Threonine>
39. Nishino H, Nishino A, Takayasu J, Iwashima A, Itakura Y, Kodera Y, Mat-suura H, Fuwa T (1990) Antitumor-promoting activity of allixin, a stress compound produced by garlic. *Cancer J* 3(1):20–21
40. Baliga MS, Shivashankara AR, Palatty PL, Dsouza JJ, Arora R (2013) Protective effect of garlic (*Allium sativum* L.) against atherosclerosis. In: Bioactive food as dietary interventions for cardiovascular disease (pp 591–607). Academic Press
41. Choi YR, Shim J, Kim MJ (2020) Genistin: a novel potent anti-adipogenic and anti-lipogenic agent. *Molecules* 25(9):2042
42. Sharifi-Rad J, Quispe C, Castillo CMS, Caroca R, Lazo-Vélez MA, Antonyak H, Cho WC (2022) Ellagic acid: a review on its natural sources, chemical stability, and therapeutic potential. *Oxid Med Cell Longevity*. <https://doi.org/10.1155/2022/3848084>
43. David AVA, Arulmoli R, Parasuraman S (2016) Overviews of biological importance of quercetin: a bioactive flavonoid. *Pharmacogn Rev* 10(20):84
44. Gopalakrishnan C, Shankaranarayanan D, Nazimudeen SK, Viswanathan S, Kameswaran L (1980) Anti-inflammatory and CNS depressant activities of xanthones from *Calophyllum inophyllum* and *Mesua ferrea*. *Indian J Pharmacol* 12(3):181
45. Poorgolizadeh E, Moghadam FH, Dormiani K, Rezaei N, Nasr-Esfahani MH (2021) Do nephrilysin inhibitors walk the line? Heart ameliorative but brain threatening! *Eur J Pharmacol* 894:173851
46. Wideliski J, Popova M, Graikou K, Glowinski K, Chinou I (2009) Coumarins from *Angelica lucida* L.-antibacterial activities. *Molecules* 14(8):2729–2734
47. Sülsen VP, Elso OG, Borgo J, Laurella LC, Catalán CA (2021) Recent patents on sesquiterpene lactones with therapeutic application. *Stud Nat Prod Chem* 69:129–194
48. PubChem. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 10088741, Austroballignan 7; [cited 2023 Oct. 8]. Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/Austroballignan-7>
49. Pettit GR, Numata A, Cragg GM, Herald DL, Takada T, Iwamoto Iwamoto C, Riesen R, Schmidt J, Doubek D, Goswami A (2000) Isolation and structures of schleicherastatins 1–7 and schleicheols 1 and 2 from the teak forest medicinal tree *Schleichera oleosa*. *J Nat Prod* 63(1):72–78
50. Kumar S, Bhandari C, Sharma P, Agnihotri N (2018) Role of piperine in chemoresistance. In: Role of nutraceuticals in cancer chemosensitization. Academic Press, pp 259–286
51. Maghzi AH, Borazanci A, McGee J, Alexander JS, Gonzalez-Toledo E, Minagar A (2011) Multiple sclerosis: pathophysiology, clinical features, diagnosis, and management. In: Neuroinflammation (pp 1–23). Elsevier
52. Hayashi T, Shinbo T, Shimizu M, Arisawa M, Morita N, Kimura M, Kikuchi T (1985) Paeonilactone-A, -B, and -C, new monoterpenoids from paeony root. *Tetrahedron Lett* 26(31):3699–3702
53. Kim SH, Lee MK, Lee KY, Sung SH, Kim J, Kim YC (2009) Chemical constituents isolated from *Paeonia lactiflora* roots and their neuroprotective activity against oxidative stress in vitro. *J Enzyme Inhib Med Chem* 24(5):1138–1140
54. Bianchi VE (2019) The anti-inflammatory effects of testosterone. *J Endoc Soc* 3(1):91–107
55. Zhabinskii VN, Drasar P, Khripach VA (2022) Structure and biological activity of ergostane-type steroids from fungi. *Molecules* 27(7):2103
56. Higano T, Kuroda M, Sakagami H, Mimaki Y (2007) Convallasaponin A, a new 5 β -spirostanol triglycoside from the rhizomes of *Convallaria majalis*. *Chem Pharm Bull* 55(2):337–339. <https://doi.org/10.1248/cpb.55.337>
57. Yang S, Fan L, Zhang R, Song C, Shi J, Wang J, Zhang Y (2023) Smilagenin induces expression and epigenetic remodeling of BDNF in Alzheimer's disease. *Phytomedicine* 118:154956
58. PubChem. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004. PubChem Compound Summary for CID 91433, 5 α -Spirostan-3 β -ol, (25R); [cited 2023 Oct. 8]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/5alpha-Spirostan-3beta-ol_-_25R

59. Chen H, Wang Z, Yang L (2012) Analysis of euphorbin in *Euphorbia helioscopia* L. and its cytotoxicity to mice lung adenocarcinoma cells (LA795). *Natural Prod Res* 26(22):2112–2116
60. Wu Y, Li F, Zhang X, Li Z, Zhang Q, Wang W, Luo K (2021) Tumor microenvironment-responsive PEGylated heparin-pyropheophorbide-a nanoconjugates for photodynamic therapy. *Carbohydr Polym* 255:117490. <https://doi.org/10.1016/j.carbpol.2020.117490>
61. Chanda J, Biswas S, Kar A, Mukherjee PK (2020) Determination of cucurbitacin E in some selected herbs of ayurvedic importance through RP-HPLC. *J Ayurveda Integr Med* 11(3):287–293
62. Yu J (2014) Ciclesonide. *Handbook of Metabolic Pathways of Xenobiotics*, 1–5
63. Oh KK (2022) Network pharmacology-based analysis of signaling pathways of an anti-osteoporotic triterpenoid from *Acyranthes bidentata* Blume root. *3 Biotech* 12(11): 312
64. Pratiwi RIA, Rahayu T, Mubarakati NJ (2020) In silico screening of *Schleichera oleosa* phytochemicals as estrogen receptors alpha inhibitors for breast cancer
65. Kähkönen M, Kylli P, Ollilainen V, Salminen JP, Heinonen M (2012) Antioxidant activity of isolated ellagitannins from red raspberries and cloudberries. *J Agric Food Chem* 60(5):1167–1174
66. PubChem. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 452242, 1-O-Galloylpedunculagin; [cited 2023 Oct. 8]. Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/1-O-Galloylpedunculagin>
67. Wang T, Lu H (2021) Ganoderic acid A inhibits ox-LDL-induced THP-1-derived macrophage inflammation and lipid deposition via Notch1/PPAR γ /CD36 signaling. *Adv Clin Exp Med* 30(10):1031–1041. <https://doi.org/10.17219/acem/137914>
68. Lee SS, Chen WC, Huang CF, Su Y (1998) Preparation and cytotoxic effect of ceanothic acid derivatives. *J Nat Prod* 61(11):1343–1347
69. Li XC, Cai L, Wu CD (1997) Antimicrobial compounds from *Ceanothus americanus* against oral pathogens. *Phytochemistry* 46(1):97–102
70. Sun B, Wu L, Wu Y, Zhang C, Qin L, Hayashi M, Liu T (2020) Therapeutic potential of *Centella asiatica* and its triterpenes: a review. *Front Pharmacol* 11:568032
71. Sulaikal Beevi I, Palavesam A. Bioactive metabolites profile of methanol flower and seed extracts of *Clitoria ternatea* (L.). <https://doi.org/10.56557/upjoz/2023/v44i203645>
72. Evans WC, Evans D (2009) Saponins, cardioactive drugs and other steroids. In: Evans WC, Evans D (eds) *Trease and evans' pharmacognosy*. Elsevier; pp 304–332
73. Fan Y, Dong W, Wang Y, Zhu S, Chai R, Xu Z, Bian Y (2022) Glycyrrhetic acid regulates impaired macrophage autophagic flux in the treatment of non-alcoholic fatty liver disease. *Front Immunol* 13:959495. <https://doi.org/10.3389/fimmu.2022.959495>

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